**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:
Harrington, *et al.*

Application No.: 09/484,331

Filed: January 18, 2000

For: **COMPOSITIONS AND METHODS FOR NON-TARGETED ACTIVATION OF ENDOGENOUS GENES**

Group Art Unit: 1632

Examiner: Shukla, R.

Attorney Docket No.: 0221-0003L

Commissioner of Patents
Washington, D.C. 20231

**DECLARATION UNDER 37 C.F.R. § 1.132**

Sir:

The undersigned, John J. Harrington, declares and states:

1. I am an inventor of the above-captioned patent application, U.S. Application No. 09/484,331, filed January 18, 2000, entitled "Compositions and Methods for Non-Targeted Activation of Endogenous Genes." I am the subject of the attached Curriculum Vitae and author of the publications shown on the list attached thereto. On the basis of the information and facts contained in these documents, I submit that I am an expert in the field of endogenous gene activation, including by non-homologous recombination, eukaryotic gene and protein expression and gene cloning and am qualified to speak on the skill and knowledge of the person of ordinary skill in these fields.

2. I have read and understand the subject matter of the above-captioned application. I have read and understand the Office Action dated April 11, 2001, rejecting claims 62–68 under 35 U.S.C. § 103. I have read and understand Treco, et al. (U.S. Patent No. 5,641,670) and DiMaio, et al. (WO 92/20784), the references used to make the rejection over Treco in view of DiMaio. I have also read and understand Chappel (U.S. Patent No. 5,272,071; EP 0779362), which was used the make the rejection over Chappel in view of DiMaio. It is my opinion, based on the scientific evidence and discussion below, that the person of ordinary skill in the art would not have been led by the information in the cited references, in view of the general knowledge in the art, to use the Treco cells, expressing an endogenous activated gene, instead of the DiMaio cells or any other recombinant cells, expressing the gene of interest by exogenous gene sequences.

3. The examiner contends that "...an artisan would have been motivated to use (the Treco) cells expressing endogenous genes that are usually silent or expressed at low levels because such cells would have provided sufficient amount of protein to test the activity and also because the protein produced would have altered the characteristics of the cell which would have helped the screening assay."

4. At the time of our filing, the artisan would not have been motivated to use the Treco cells to produce protein for drug screening because making a homologously recombinant cell expressing a given protein carried with it several significant disadvantages over the standard

recombinant approach to protein expression, i.e., expression from an exogenous coding sequence. The standard recombinant approach could provide protein in a short period of time relative to the time required using the Treco approach. It was much less arduous than the Treco approach with respect to the manipulations necessary to produce protein. It provided much greater flexibility than the Treco approach for protein production and drug testing. In short, with standard recombinant techniques, the process for producing proteins was straightforward and efficient. Accordingly, if the person of ordinary skill in the art had desired to screen a compound against a specific protein, but found that there were no sufficient natural expressors available, the person of ordinary skill in the art would have used standard recombinant gene expression, given the choice between using the Treco methods and standard recombinant expression methods.

5. The first advantage of using standard recombinant DNA methods to express a gene of interest is that any coding sequence from a gene of interest could be directly inserted into a variety of pre-existing expression vectors. Many suitable expression vectors were even commercially available. Thus, any given gene could simply be inserted into the cloning site of a pre-existing expression vector. The artisan did not have to create, *de novo*, an expression vector to clone the gene into. For example, if one had desired to express EPO in mammals, they would simply have cloned it into a pre-existing mammalian expression vector. If one had desired to express EPO in insects, one would simply have cloned it into a pre-existing insect expression vector. The fact that vectors were available that could express any desired gene cloned into them allowed the artisan to proceed from gene sequence to protein expression in a very short timeframe relative to the time involved using the Treco methods.

In contrast to using standard recombinant methods of gene expression by cloning exogenous coding sequences into a pre-existing expression vector, to express a protein using the Treco methods, the gene had to be identified and characterized. For example, once a gene coding sequence was known, at a minimum, the sequence and structure of exon I had to be determined, a process requiring the mapping of exon/intron boundaries in genomic DNA. In addition, the artisan would have had to clone appropriate exon sequences and splice sites into an expression vector. The artisan also would have had to clone appropriate targeting sequences onto the gene activation vector. Again, this process would have required the artisan to obtain additional information relating to the genomic organization of the gene of interest. Importantly, all of this work had to be done for each gene to be expressed. Thus, the artisan, without a suggestion to do otherwise, would not have been motivated to produce protein for drug screening using the Treco method.

The second advantage of using standard recombinant DNA methods to express protein is that expression vectors allow the artisan to produce protein in relatively short time periods. These could be used to produce protein transiently, a process that takes just a few days. Recombinant methods could also be used to produce protein *in vitro*, a process that takes just a few hours. Recombinant methods could also be used to produce stable transfectants producing sufficient protein. This process could require several weeks. The Treco method, however, could easily require months to produce sufficient protein.

A third advantage of standard recombinant methods for protein expression is that these methods result in a much higher probability that an insertion event will be productive, i.e., that an integration event will result in expression of the desired gene. The Examiner recognizes, and Treco teaches, that the vast majority of insertion events using the Treco homologous recombination methods are non-productive. Accordingly, many more cells must be screened to find an expressor, much less a sufficient expressor. The artisan might not recover a single positive expressor from the same number of transfected cells used in the standard recombinant DNA approach.

Another significant advantage of standard recombinant DNA expression vectors is that a desired gene can be expressed in virtually any species using routine and straightforward methods. Using the Treco methods, expression of a drug target could only be carried out in cells that encode an endogenous copy of the gene. For example, if the artisan desired to express a human protein for drug screening, expression would have to be carried out in human cells using the Treco method since only human cells contain endogenous human genes. On the other hand, use of the cloned gene would have allowed the artisan to use human cells, mouse cells, rat cells, hamster cells, insect cells, yeast cells, bacteria, or any other cell to express the protein of interest. In fact, by working with the isolated gene, the artisan could quickly survey a variety of cell types to identify cells that are best suited for the particular drug screen of interest. Thus, use of the isolated gene provided the artisan with far more alternatives for producing high levels of the protein of interest. This, in turn, gave the artisan a higher probability of success.

6. Based on the above considerations, it is my opinion that, without a suggestion to do otherwise than use the standard recombinant DNA approach used by DiMaio, the person of ordinary skill in the art would not have been motivated to use the Treco methods because they were far more laborious and had no advantages.

John J. Harrington, Ph.D.

8/9/01
Date

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## EDUCATION

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|---|---|
| 1994-1998 | Case Western Reserve University, Cleveland, OH<br>Post-doctoral Research in Human Molecular Genetics |
| 1989-1994 | Stanford University, Stanford, CA<br>Ph.D. in Cancer Biology |
| 1985-1989: | University of California, San Diego, San Diego, CA<br>B.A. in Biochemistry and Cell Biology (Cum Laude) |

## RESEARCH EXPERTISE

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| Protein Biochemistry | Protein purification, gene overexpression, assay design, enzyme kinetics, protein structure-function analysis, identification of novel enzymatic activities |
| Molecular Biology | Molecular gene cloning, PCR, cDNA and genomic library construction, nucleic acid enzymology, vector construction, Southern and Northern Blot analysis |

## RESEARCH AND MANAGEMENT EXPERIENCE

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| 1995-present | Executive Vice President, Chief Scientific Officer, and Director, Athersys, Inc. Responsible for directing all research and development activities at the Company, including development of the Company's proprietary technology platforms. Along with the Board of Directors, responsible for oversight on all matters related to Company operations, including budgetary, legal, and strategic issues. |
| 1994-1998 | Post-doctoral Research Associate, Case Western Reserve University<br>Constructed Human Artificial Chromosomes. Developed assays for characterization of human artificial chromosomes and the genetic elements required for chromosome function. |

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| 1989-1994 | Graduate Research Associate, Stanford University.<br>Dissertation Research: Purification, characterization, and molecular cloning of the FEN-1 family of structure-specific endonucleases.<br>Additional Research: Developed cellular and cell-free assays for V(D)J recombination.<br>Thesis Advisor: Dr. Michael Lieber |
| 1988-1989 | Research Assistant, Scripps Clinic and Research Foundation.<br>Purification and characterization of Protein C Inhibitor from human plasma.<br>Principal Investigator: Dr. John Griffin |

## MEETINGS AND ABSTRACTS

List available upon request

## PUBLICATIONS

**Harrington, J. J.**, C. L. Hsieh, J. Gerton, G. Bosma, M. R. Lieber (1992). Analysis of the Defect in DNA End Joining in the Murine scid Mutation. *Mol. Cell. Biol.* 12(10): 4758-4768.

**Harrington, J. J.** and M. R. Lieber (1994). The Characterization of a Mammalian Structure-specific Endonuclease. *EMBO J.* 13(5): 1235-1246.

**Harrington, J. J.** and M. R. Lieber (1994). Functional Domains within FEN-1 and RAD2 Define a Family of Structure-specific Nucleases: Implications for Nucleotide Excision Repair. *Genes and Development* 8(11): 1344-1355.

**Harrington, J. J.** (1994). The Characterization of the FEN-1 Family of Structure-specific Endonucleases: Implications for DNA Replication, Recombination, and Repair. Ph.D. Dissertation, Stanford University.

Hiraoka, L., **J. J. Harrington**, D. S. Gerhard, M. R. Lieber, and C. L. Hsieh (1995). Sequence of Human FEN-1, a Structure-Specific Endonuclease, FEN-1, and Chromosomal Localization in Mouse and Human. *Genomics* 25: 220-225.

**Harrington, J. J.**, and M. R. Lieber (1995). DNA Structural Elements Required for FEN-1 Binding. *J. Biol. Chem.* 270(9): 4503-4508.

X. Li, J. Li, **J. J. Harrington**, M. R. Lieber, P. M. J. Burgers (1995). Lagging Strand DNA Synthesis at the Eukaryotic Replication Fork Involves Binding and Stimulation of FEN-1 by PCNA. *J. Biol. Chem.* 270(38): 22109-22112.

Harrington, J.J., G. Van Bokkelen, R.W. Mays, K. Gustashaw, H.F. Willard (1997). Formation of de novo centromeres and construction of first-generation human artificial microchromosomes. *Nature Genetics* 15: 345-355.

PATENTS

U.S. patent # 5,869,294. Method for stably cloning large repeating DNA sequences (1999); **Harrington; J. J.**, Van Bokkelen; G.B., and H.F. Willard.

U.S. patent # 5,874,283. Mammalian flap-specific endonuclease (1999); **Harrington, J. J.**, Hsieh; C.L., M.R. Lieber.

U.S. patent # 5,695,967. Method for stably cloning large repeating units of DNA (1997); Van Bokkelen; G.B., **Harrington; J. J.**, and H.F. Willard.

REFERENCES

Available upon request.